

PATENT SPECIFICATION

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DRAWINGS ATTACHED

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(54) METHOD AND APPARATUS FOR FORMING ELECTROPHORESIS APPARATUS AND THE LIKE

(71) I, FRANKLIN RAY ELEVITCH, a citizen of the United States of America, of 430 Nevada Street, Palo Alto, California, United States of America, do hereby declare 5 the invention for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:

10 This invention relates to thin film diffusion analysis apparatus for use in conducting electrophoresis (and subsequent fluorometry or densitometry) and for use in conducting radial diffusion operations, and 15 to methods of making such apparatus.

In general the present invention relates to various modifications and improvements in the disclosures of the specifications of my earlier patents Nos. 1,211,008; 1,211,009 20 and 1,211,010.

According to the present invention there is provided a method of forming apparatus for diffusion analysis which comprises providing a base having a surface which is 25 adherent to aqueous, permeable gels, applying a layer of such gel to the adherent surface, and applying a cover to the layer of gel with one surface of the cover in contact with the layer of gel, said contacting surface 30 being coated with a normally solid, thermoplastic essentially hydrocarbon, hydrophobic material which is non-adherent to said gel. There is also provided apparatus for diffusion analysis comprising an impermeable base member, a cover member, and a layer of aqueous, permeable gel between the members, and adherent to the base member, the inner surface of the cover member being uniformly coated with a normally 35 solid thermoplastic, essentially hydrocarbon, hydrophobic material which is in surface contact with the gel and to which the gel is non-adherent.

The base may be provided in the form of 40 a multiplicity of segments or as a continu-

ous strip, such segments or strip being supplied at a film forming station at which the layer of gel is applied in liquid or semi-solid form and the applied film being caused to solidify before application of the cover 50 to the solidified film.

Certain forms of the invention are illustrated by way of example in the accompanying drawings, in which:

Figure 1 is a perspective view of one 55 form of cover and base for apparatus in accordance with the present invention;

Figure 2 is a section taken along the line 2-2 of Figure 1 but with the base applied to the cover: 60

Figure 3 is a perspective view of the base of the apparatus of Figure 1 separated from the cover and with a film of gel adhering to it:

Figure 4 is a cross section taken along 65 the staggered line 4-4 of Figure 3:

Figure 5 is a diagrammatic drawing of a semicontinuous method for manufacturing apparatus in accordance with the invention:

Figure 6 is a view in vertical section 70 through a thin film device as made by the method of Figure 5:

Figure 7 is a diagrammatic drawing showing a continuous method of manufacturing thin film devices: 75

Figure 8 is a view taken along the line 8-8 of Figure 7 showing in elevation the roller used to fabricate thin film devices;

Figure 9 is a fragmentary view showing an alternative to the apparatus of Figures 80 7 and 8; and

Figure 10 is a view in vertical section of a stack of devices in accordance with the invention.

In the later described apparatus of my 85 invention the film of permeable gel which provides the medium for migration of specimens, reagents and the like is an aqueous gel and it is preferably a thin film, usually not more than one millimeter thick and 90

preferably not more than about 0.5 millimeter thick. However, in some embodiments of the invention the film of gel may be thicker. This film is permeable to reagents such as those mentioned hereinafter and to biological materials such as proteins and blood serum, such that the selected reagents, specimens or reaction product will migrate by capillary effect or under an applied voltage.

The thin films of gel are also preferably uniform in thickness, and preferably do not depart by more than about 10% from the average thickness.

15 The thinness and uniformity of these films are highly advantageous. The gel thinness permits rapid migration under an applied voltage of 10-15 volts per centimeter or more without the need for applied cooling.

20 and without destructive heat build-up which would dry the gel or denature the material migrating into the film, such as proteins. This rapid migration without elaborate cooling equipment and without destructive heat build-up is advantageous in electrophoresis because it permits the use of high voltages with attendant high speed of separation, and without applied cooling, and it also permits the use of inexpensive equipment.

25 which can be supplied to the customer ready for use and which is inexpensive enough to discard after a single use. In the radial diffusion technique thin films are advantageous because they allow the use of

30 minimum quantities of expensive reagents such as antisera and they also provide sharp end points. In all cases uniformity of film thickness is advantageous because results are more reliable.

40 Advantages of thin films of gel have been noted heretofore. See Wadsworth, "A Slide Microtechnique for the Analysis of Immune Precipitates in Gel", Int. Arch. Allergy, vol. 10, pp 355-360 (1957). However, prior to
45 my inventions practical apparatus employing thin films of gel which lends itself to manufacture in a form ready for use, which can be stored for extended periods of time without deterioration, which is simple to
50 use and which is sufficiently inexpensive that it can be used once and discarded, has not generally been available.

In Figures 1 and 2 one form of apparatus is shown which provides these advantages.

55 Referring now to Figures 1 and 2, a complete mould is there shown which comprises a cover 10 and a base 11 which may be made of the same or different materials such as polystyrene, polyethylene or polypropylene. Polystyrene is a preferred material because of its superior machining and fabrication qualities and because it is transparent and non-fluorescent. Non-fluorescence is of particular importance in applications where

60 65 Fluorometry is used because a fluorescent

material is incompatible with this technique. For example, where the separated proteins are stained with a dye which fluoresces, background fluorescence of the material of the base would be incompatible. Polyethylene and polypropylene meet these requirements but their machining and/or fabrication qualities, although acceptable, are not as good as those of polystyrene. Other materials of construction may be used. The 75 cover 10 preferably has substantial thickness and is rigid while the base 11 is preferably thin and flexible. Transparent materials such as polystyrene, polypropylene and polyethylene are preferred although 80 where reflectance methods of fluorometry are used an opaque material may be used. However, even in those cases transparent materials of construction are preferred because, among other things, they permit 85 visual inspection to detect bubbles or voids.

Visual inspection to detect bubbles or voids. The base 11 preferably has a flat inner surface. The cover 10 is provided with a rim formed by end walls 12 and side walls 13 which define a mould cavity 14 and which determine the depth of the mould cavity and therefore the thickness of the resulting film of gel. The rim 12, 13 provides a face 16 to which a pressure sensitive adhesive is applied as explained below. The inner surface of the cover 10 is formed with risers 19 and well forming projections 24a and 24b. As shown in Figure 2, the risers 19 have the same height as the rim 12, 13 and they are intended to contact the base 11 when the apparatus is assembled. The risers 19 and the rim 12, 13 determine the depth of the mould cavity and therefore the thickness of the film of gel. They are formed with accuracy for this purpose so that the film of gel has a uniform thickness. Preferably the mould cavity depth and therefore the film thickness are less than 1 mm., preferably about 0.3 to 0.5 mm. 100 105 110

The well forming projections 24a and 24b, as appear in Figure 2, preferably terminate short of the base 11 so that the resulting sample receiving wells do not extend to the base. This is shown in Figure 4 and it is advantageous because, if the wells extend through the base, undercutting of solution placed in the wells may occur with resulting fuzzy or ambiguous results. In Figures 3 and 4 a layer of gel 28 is shown adhering to the base 11, such film being formed with sample receiving wells 29a and 29b and with channels 31 formed by the well forming projections 24a and 24b and the risers 19, respectively. The cover 10 is provided with filling and venting openings 26. Caps (not shown) may be provided to close the vents but preferably the vents 26 are heat sealed to close them. 110 115 120 125

In Figure 4 the cover 10 is shown in broken line, the purpose being to illustrate 130

how the projections 24a and 24b and the risers 19 remain within the wells 29a and 29b and the channels 31 until such time as the base 11 with the adhering film of gel 28 is stripped from the cover (see below). This is an important advantage of the construction of my invention because the gel, being aqueous (about 90% water), will leak into the wells 29a, 29b, also into the channels 31. Therefore, without the projections 24a, 24b, and risers 19, the wells and channels would be filled and their integrity would be destroyed. By reason of the projections 24a, 24b and the risers 19 their integrity is preserved during long periods of storage, and by reason of the edge sealing described below, the integrity of the entire film against evaporation is also preserved during long periods of storage.

As described in earlier applications above mentioned the gel 28 is adherent to the base 11 and is non-adherent to the cover 10 such that, when it is desired to separate the base it may be stripped from the cover with the film of gel 28 adhering to the base and completely separated from the cover, to which none or a negligible quantity of the film adheres. A release agent is used to prevent adherence of the gel to the cover 10, which may therefore, be made of the same material as the base 11.

In accordance with the present invention an improved type of release agent and an improved method of applying the release agent are provided for the cover 10. For this purpose a solution of paraffin wax in a volatile solvent is applied to the cover 10 by dipping, spraying or other means. A suitable wax, when the cover is made of polystyrene having a softening point of about 90°C, is a refined paraffin wax having a melting point (AMP) of 128/130°F; a colour (Saybolt, ASTM D156) of + 30; an oil content (ASTM D721) = 0.3%; a penetration at 77°F and 100°F (ASTM D134) = 17 and 105, respectively; and a viscosity at 180°F and 210°F (SSU, ASTM D2161) = 41.6 and 38.2, respectively. Any other paraffin wax, and in fact any other thermoplastic, essentially hydrocarbon, hydrophobic material may be used which will adhere to the cover, which is non-adherent to an aqueous gel such as agarose gel, which is solid at temperatures up to about 70°C and which melts substantially below the melting point of the cover material. A solution, e.g., in low boiling petroleum ether (b.p. = 36-40°C), is applied by any suitable means as by dipping or spraying. It does not matter that the top portion of the cover is also coated inasmuch as the amount wasted is not great and does not interfere with subsequent use of apparatus. If the solution is applied by dipping, the cover may be allowed to drain. If the solution is

applied by spraying, it is applied so as to provide a uniform coating of wax on the surface.

Next, the cover is dried by air drying at ambient temperature, by means of applied heat such as a current of hot air or by any other suitable means. This leaves a very thin coating of wax on the inner surface of the cover. This coating is uniform but contains small voids. To eliminate these voids, the coating of wax is melted and glazed by a mild application of heat, as by means of a current of hot air, by holding over a hot plate or otherwise. The temperature and time of heating are such that glazing of the wax coating occurs but without damage to the material of which the cover 10 is constructed. By this means the coating on the cover is made uniform and voids are filled in by coalescence.

Before or after this wax coating operation, preferably afterwards, a pressure sensitive adhesive such as a rubber cement is applied to the face 16 of the rim 12, 13 and/or to the corresponding marginal area of the base 11. Preferably the pressure sensitive adhesive is applied to the face 16 of the rim 12, 13 of the cover 10. Also heat sealing may be employed instead of adhesive.

Next the cover 10 and base 11 are assembled as shown in Figure 2 and sufficient pressure is applied, as by means of a platen press, to adhere the base firmly to the cover. This pressure brings the face 16 of the rim 12, 13 of cover 10 into firm, uniform contact with the cover 11 and it also brings the risers 19 into firm contact with the cover 11, so that the space between the cover 10 and base 11, hence the thickness of the resulting film of gel is uniform. Then a melted gel is introduced through one of the openings 26, air being vented through the other opening. A suitable gel formulation consists of 10% by weight of sucrose and 1 to 1.2% by weight of agarose dissolved in an electrolyte solution. The electrolyte solution may be an aqueous solution of sodium barbital and HCl having a pH of 8.6. Many such aqueous gels and aqueous electrolytes are well known. Other gelling agents such as starch, agar or acrylamide may be used in place of agarose. A gel formed in accordance with the complete formulation given above is advantageous because it is transparent and non-fluorescent and it has good properties for migration of materials by capillary effect and/or under a voltage gradient as in electrophoresis.

Preferably the gel is degassed before it is placed in the mould. It has been found that, on occasion, the film of gel forms gas bubbles, especially if the apparatus is stored in a hot climate or in hot surroundings. Such degassing is readily accomplished by

placing the gel in a vacuum vessel, applying sufficient heat to melt it and subjecting it to a vacuum of about 28 inches of mercury for about 5-10 minutes. The gel may be stirred 5 to accelerate degassing.

Preferably also the gel has incorporated in it a very small amount, for example about 0.05 to 0.2%, by weight of a polyvinyl alcohol. A suitable polyvinyl alcohol is a 10 99% hydrolyzed (i.e., 99%) of the acetyl groups of polyvinyl acetate are removed by hydrolysis), having a viscosity of 28-32 centipoises. This is used as a 4% aqueous solution. The inclusion of polyvinyl alcohol 15 considerably improves the release properties of the gel from the cover.

After the mould (which is in a press, see above) has been filled with gel (which is supplied in warm, molten condition), as 20 indicated by an overflow from the venting spout 26, the caps 27 are applied to the spouts 26 or the spouts 26 are heat sealed and, after the gel has cooled and solidified, the apparatus is ready for storage, shipment and use.

In use, as described in my earlier application No. 1,211,008, the base 11 is peeled from the cover and carries with it a thin, uniform film of gel. This film of gel is 30 formed with troughs or channels 31 formed by the risers 19 and sample receiving wells 29a and 29b formed by the well forming projections 24a and 24b. By reason of retention of the cover 10 and its projecting 35 risers 19 and well forming projections 24a and 24b until the time of use the integrity of the troughs and wells is preserved. Specimens are placed in the wells 29a and/or 29b. If separation by electrophoresis is to be 40 carried out, the base 11 with its adhering film of gel is placed in a suitable electrophoresis apparatus and is subjected to a suitable voltage gradient to effect separation of proteins. By reason of the uniformity 45 of the layer of gel, sharp separations are produced and because of the thinness of the gel, high voltage gradients (e.g., 10 to 15 volts per centimeter) can be applied without external cooling. Rapid separations 50 are effected, e.g., in one-half hour to one hour. The apparatus is then placed in suitable analytical equipment, after suitable staining and other processing. The analytical equipment may be a fluorometer or a densitometer to measure quantitatively the separated proteins. If the apparatus is to be 55 used in the radial diffusion-precipitin technique, the body of the gel will have been impregnated with a suitable reactant such as 60 an anti-serum and specimens, e.g., blood serum, will be placed in the sample receiving wells and will diffuse outwardly and will form rings (which can be made visible or more readily visible by staining) by reaction with anti-serum. Measurement of the

diameters of these rings and of the diameters of rings produced by control specimens of known material will provide a quantitative measure of the material to be measured.

In the foregoing description, the film of 70 gel is described as having sample receiving wells formed in it by well forming projections. In another embodiment of the invention the film of gel is made without such wells, in which case the cover 10 is formed 75 without the projections 24a, 24b and if desired, without the risers 19. Specimens may be applied to the plain surface of the film of gel and caused to migrate radially outwardly as in the radial diffusion technique, or to migrate directionally as in electrophoresis. Wells may be formed in the film after the cover 10 is removed, or the cover 10 may be formed with holes which are sealed during storage, then opened at the 80 time of use to permit introduction of specimens. Alternatively, a template with holes in it may be placed over the exposed film (after removal of the cover) and specimens 85 introduced through the holes.

Apparatus such as that shown in Figures 1 and 2 may be made in a semi-continuous way. Referring to Figure 5 a continuous tape 40 supplied from a roll (not shown) is provided, such tape being, for example, of 90 polystyrene and having the same thickness as the base 11 in Figure 1, for example about 0.005 inch. This tape may be passed through a tank 41 filled with a cleaning solvent such as isopropyl alcohol although 95 such may be unnecessary. The tape is guided around a guide roller 42 and is dried by means of a current of hot air (not shown). The tape then passes along a table 43 through a film forming station A and pauses 100 at timed intervals at that station while a mould 44 is lowered into the tape. The mould 44 has a rim which forms a mould cavity 46 having the desired depth and horizontal shape and dimensions. The 105 mould 44 is shown, for simplicity, without well forming projections and risers such as 24a, 24b and 19 but it may be provided with them. A rod 47 connected to a suitable 110 operating mechanism (not shown) lowers the mould from the raised position shown in broken lines to the lowered operating positions shown in solid lines in contact with the tape. During this pause or dwell and 115 while the mould is in the firm contact with the tape, a gel is injected from a suitable source (not shown) through one of the spouts 48. Air is vented through the other spout along with excess gel. The gel is introduced 120 in liquid or semi-solid condition and the dwell at station A is long enough to permit such filling operation and to allow the gel to solidify. If desired a current of cold air 125 may be applied to the mould 44 and/or to the under surface of the table 43, or cooling 130

liquid may be passed through passages (not shown) in the mould and/or table to hasten solidification of the gel. The mould is then tilted to the angular position shown in dotted lines to release it from the solidified film of gel and it is then raised to the horizontal position, also shown in dotted lines, to allow the tape to move another increment. Preferably the interior of the mould 5 will have been coated with a suitable release agent unless the material of construction is inherently non-adherent. Suitable release agents are silicone release agents and paraffin wax as described above. The 10 tape 40 also pauses at an applicator station B where a mould cover 10 identical with that shown at 10 in Figure 1 may be applied to the film of gel 50. This application may be manual or it may be done automatically 15 by a suitable mechanism (not shown) during a dwell in the tape movement. The mould cover will have been coated with paraffin wax, as described above. Also the face 16 of the rim of the cover (and/or a corresponding marginal area of the tape) will have been coated with a pressure sensitive adhesive. As an aid, especially where manual application of the mould cover is employed, the upper face of the tape 40 may be printed 20 at intervals with an outline of the rim of the mould.

Downstream from station B the tape is severed by a knife (not shown) to produce a complete package 55 (Figure 6) composed of a base 56 (the severed portion of tape 40), a thin film of permeable aqueous gel 50 and a cover 57 applied at station B and which laps over the edges at 57a around the entire periphery of the film 50. This 25 cover also extends into wells 59 and channels 60 formed in the film by the mould 44. When it is desired to use the apparatus 55, a segment of the overlap 57a is lifted (being pried loose with a blade if necessary) and 30 is stripped from the film 50, which is then ready for use. As in the case of the cover 10 in Figures 1, 2 and 4, the cover 57, by projecting into wells 59, etc. preserves their 35 integrity during storage.

Referring now to Figures 7 and 8 equipment is there shown for continuous fabrication of apparatus of the present invention. This equipment comprises a continuous tape 40, a tank 41 (not shown) and solvent 45 and a guide roller 42 as in Figure 5. A block 70 is shown which is formed with passages 70a for circulation of cooling fluid. The block 70 has a concave portion at 71 50 and a convex portion at 72. A roller is provided at 73 which is nested in the concave portion 71 of the block 70 and at the lower end of a sloping portion 74. An applicator such as a nozzle is provided at 75. Referring more particularly to Figure 60 8, at opposite sides of the sloping portion 65 74 of the block 70 and at opposite ends of the roller 73 retainer plates 76 are provided. The roller 73 is provided with trunnions 77 by which the roller is rotatably supported in a suitable framework (not shown). The 70 roller 73 is provided with sprocket teeth 78 which are intended to engage mating holes in the tape 40 so that the tape moves synchronously with the rotary motion of the 75 roller 73. Alternatively, engagement of the 80 tape and roller may be frictional and without a sprocket arrangement. The roller 73 is shown formed with mould units 79, each of which has a depth equal to the desired 85 thickness of the film of gel, that is preferably not more than 1 mm and most preferably about $\frac{1}{2}$ mm or less. Each mould unit comprises ribs or risers 80 and well forming projections 81a, 81b. The ribs 80 extend to the periphery of the roller and therefore contact the tape 40. The well forming projections 81 preferably terminate short of the periphery of the roller and therefore do not contact the tape 40. Any desired 90 number of mould units 79 may be provided, 95 four being shown on one face of the roller in Figure 8, there being four on the other face, although any number desired may be used.

In operation the tape 40 is moved continuously through the apparatus by reason of engagement of sprocket 78 with holes in the tape (or by frictional engagement). Such movement is preferably at a uniform rate such that liquid gel material may be supplied 100 at a uniform rate by means of the applicator 75 to maintain an adequate pool of liquid or semi-solid gel in the well formed by the sloping section 74 of block 70 and the retainer plates 76. If need be, heat may be applied to this pool to maintain it in adequately fluid condition. As each mould unit 79 rotates into contact with this pool of gel it will be filled with gel and will rotate into the cooled sector of its cycle. Cold 105 fluid circulating through the passages 70a in the block 70 and, if desired, through similar passages (not shown) in the roller 73 acts to solidify the liquid gel thus trapped in each mould unit 79. During transit along 110 the concave portion 71 of the block 70 this trapped body of gel solidifies. There results from this mode of operation a multiplicity of films of solidified gel each imprinted with wells and troughs formed by the well forming projections 81 and the ribs 80. The convex portion 72 of the block 70 provides a reverse bend which assists in separation of each film of gel from the roller. These films 115 of gel, supported on the tape 40, pass along a table 81 and, at an appropriate point, a cover such as that shown in Figure 1 is applied manually or automatically by suitable means (not shown). Subsequently a knife 83 is used to sever the tape 40, result- 120 125 130

ing in the production of individual packages such as that shown at 55 in Figure 6.

In Figure 8 the cylinder 73 is shown with longitudinal segments 85 separating the mould units 79. Alternatively, these segments may be omitted, i.e., the dotted lines 86a in Figure 8 represent continuations of the side walls 86 of the respective mould units 79. In such case, the cylinder 73 is formed with one or more (two being indicated in Figure 8) continuous circumferential recesses with ribs 80 and well forming ribs 81a, 81b located therein. As a result, the product issuing from the cylinder at the convex portion 72 of block 70 comprises not only a continuous tape 40 but also a continuous film (or two or more continuous films) of gel with wells (and such other impressions as desired) formed thereat in at intervals. Covers are applied and the continuous, tape-like laminar product may be severed into segments as and when desired, or it may be wound up in continuous form into a roll for subsequent use or severing.

Further, wells and troughs need not be formed in the film. In such case, if sample receiving wells are needed, they may be formed subsequently. Also specimens may be applied to plain gel film, that is to film formed without an imprinted pattern of wells or the like in the manner described above.

Referring now to Figure 9, an alternative to the cylinder 73 of Figures 7 and 8 is shown. This alternative, generally designated as 100, comprises an endless member 101 extending about wheels or sprockets 102 and 103 (with intermediate wheels or sprockets if needed). A table 104 has a downwardly sloping section 105 at the input end (the lower reach of the member 101 moving from left to right), and an upwardly sloping section 106 at the other end. The endless member is formed with separate mould sections like sections 79, or with a continuous mould section, with or without well forming projections as at 81a, 81b and risers as at 80 as desired. That is to say, the endless member 101 is, in effect, a transformed cylinder, lengthened out and having an operative, mould forming configuration. Tape is shown at 40, a supply nozzle at 75 and film 80a on the tape is also shown. An advantage of the apparatus of Figure 9 is that it provides an elongated cooling path wherein the moulding element (endless member 101) has sufficient length to allow solidification of the film of gel. Cooling fluid may be passed through the endless member 101 and/or the table 104 and/or a current of cold air may be applied to hasten solidification of the gel.

In those embodiments of the invention in which the film is plain and is not imprinted

with wells and the like whose integrity must be preserved, the film of gel, after being formed, is covered with a non-adherent sheet material such as polyethylene film coated with a release agent such as paraffin wax, and the resulting packages may be stacked one on top of the other. Edge sealing to prevent evaporation of the gel may be provided by overlap of the overlying sheet material, by heat sealing such overlap, etc., or by packaging the film in a tight container which seals the exposed edges.

A packaged product formed in this manner is shown in Figure 10. The package is generally designated as 110 and it comprises a bottom closure 112, side walls 113 and a top closure 114. The parts 112, 113 and 114 may be integral and they may be made of polyethylene, polystyrene or any other suitable material. Within this container are a multiplicity of units each comprising a base 115 and a film of permeable gel 116. The top surface of each base is adherent to the gel and the bottom surface is non-adherent to the gel. For example the base members 115 may be made of polystyrene, which is adherent and the bottom surface of each polystyrene base may be coated with a release agent such as paraffin wax. Therefore, when the package is opened, the top base member 115 may be stripped from the stack with the superimposed film of gel adhering to it but the layer of gel beneath being non-adherent. The arrangement shown in Figure 10 with each base 115 beneath and the adhering film of gel above, may be reversed, that is the top surface of each base 115 may be made non-adherent and the bottom surface may be made adherent. In effect, in this variant, the package 110 in Figure 10 is merely inverted so that 112 becomes the top and 114 becomes the bottom.

The extreme thinness and uniformity of the film of gel are advantageous not only for the reasons previously stated but because the steps involved, moistening, drying, curing, etc. can be accomplished very quickly. If thick layers of gel are employed, the time required for these steps may be prohibitive.

In general optimum thickness and optimum uniformity can be determined by criteria such as those mentioned immediately above and elsewhere above. I have found that thickness less than about 1 mm should be used, preferably not more than about 0.5 mm; and that departures from average thickness preferably do not exceed about 10%, most advantageously not by more than about 7%.

In my copending divisional Application No. 29488/72 (Serial No. 1338580) I describe and claim apparatus for diffusion analysis comprising a stack of superposed units each comprising a base member and

a layer of aqueous permeable gel in adhered contact with one surface of the base member, the other surface of each base member which is in contact with an adjacent layer 5 of gel being non-adherent to such gel.

In my aforementioned Patent No. 1,211,008 I describe and claim, inter alia, apparatus for diffusion analysis comprising an impermeable substantially flat base, a 10 film of substantially uniform thickness of permeable gel not more than 1 mm. thick having one surface in continuous contact with and adhering to one surface of the base, and a cover having a surface in continuous 15 contact with the other surface of the film of gel and being in edge contact with the base completely to enclose and to seal the film, the film of gel being substantially non-adherent to the cover to permit removal of the base and gel adherent thereto from the cover, and a method of making a diffusion analysis device comprising filling 20 a mould having a first, base section and a second, cover, mould section in edge contact with the mould base section with a medium suitable for diffusion analysis, allowing the medium to harden to form a permeable gel which adheres to the mould base section but not the mould cover section, and stripping the mould base section 25 from the mould cover section to form a base having a thin film of gel adhering thereto, the film having a substantially uniform thickness of not more than 1 mm.

35 **WHAT I CLAIM IS:—**

1. A method of forming apparatus for diffusion analysis which comprises providing a base having a surface which is adherent to aqueous, permeable gels, applying a layer 40 of such a gel to the adherent surface, and applying a cover to the layer of gel with one surface of the cover in contact with the layer of gel, said contacting surface being coated with a normally solid, thermoplastic essentially hydrocarbon, hydrophobic material which is non-adhering to said gel.
2. A method according to claim 1, wherein the hydrocarbon material is paraffin 50 wax.
3. A method according to claim 2, wherein the paraffin wax is applied in solution in a volatile solvent.
4. A method according to claim 3, 55 wherein the applied coating of wax, after evaporation of solvent, is heat glazed.
5. A method according to claim 1, 2, 3 or 4, wherein the cover is formed on its contacting surface with well-forming projections that project into the layer of gel 60 and form sample-receiving wells therein.
6. A method according to any preceding

claim, wherein the layer of gel does not exceed one millimeter in thickness.

7. A method according to any preceding 65 claim, wherein the layer of gel contains a polyvinyl alcohol.

8. A method according to any one of claims 1 to 7 wherein the base is provided in the form of a multiplicity of segments or 70 as a continuous strip, such segments or strip are supplied at a film forming station at which the layer of gel is applied in liquid or semisolid form, and the applied film is caused to solidify before application of the 75 cover to the solidified film.

9. A method according to claim 8, 80 wherein the base is supplied as a continuous strip and the gel layer applying and covering steps occur at different locations, there being a dwell in the movement of the strip at each location to permit the layer application and cover application steps to be carried out in sequence.

10. A method according to claim 8, 85 wherein the base is supplied as a continuous strip and the film of aqueous gel is applied by a continuously operating applicator.

11. Apparatus for diffusion analysis comprising an impermeable base member, a 90 cover member, and a layer of aqueous, permeable gel between the members and adherent to the base member, the inner surface of the cover member being uniformly coated with a normally solid thermoplastic, essentially hydrocarbon, hydrophobic material which is in surface contact with the gel and to which the gel is non-adherent.

12. Apparatus according to claim 11, wherein the inner surface of the cover 100 member is coated with paraffin wax.

13. Apparatus according to claim 11 or 12, wherein the cover member is formed with well-forming projections which project into the gel to form sample-receiving wells. 105

14. Apparatus according to claim 11, 12 or 13, wherein the layer of gel does not exceed one millimeter in thickness.

15. Apparatus according to claim 11, 12, 13 or 14, wherein the gel contains a 110 polyvinyl alcohol.

16. A method of forming apparatus for diffusion analysis substantially as herein described with reference to the accompanying drawings.

17. Apparatus for diffusion analysis constructed and arranged substantially as herein described with reference to the accompanying drawings.

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115

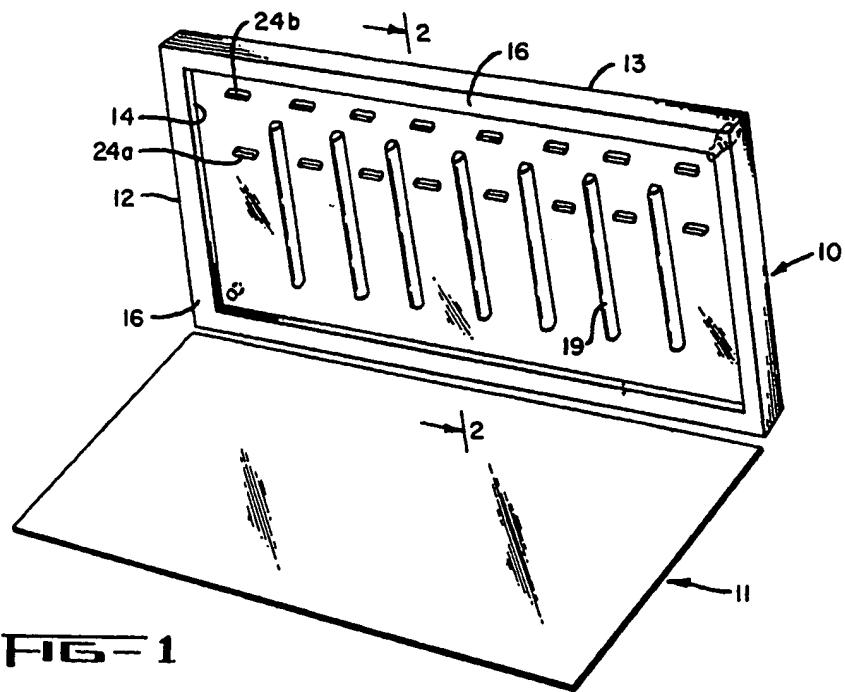


FIG-1

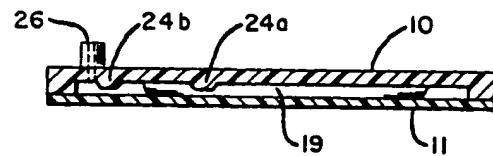


FIG-2

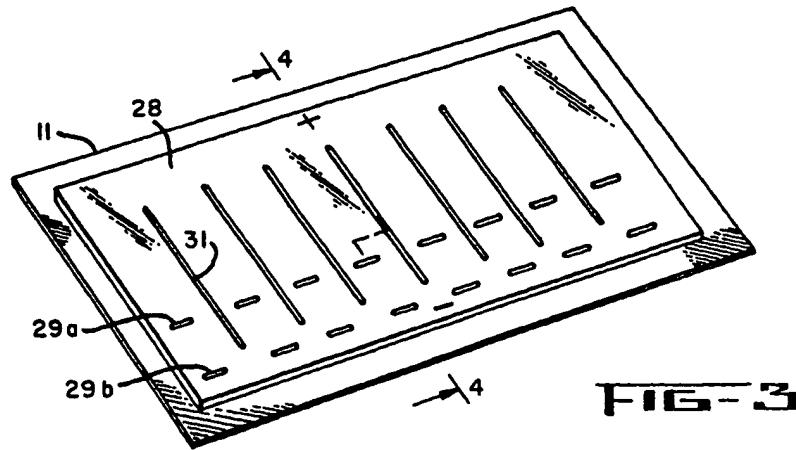


FIG-3

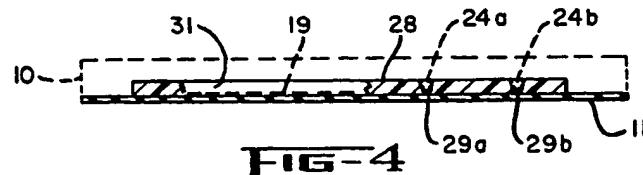


FIG-4

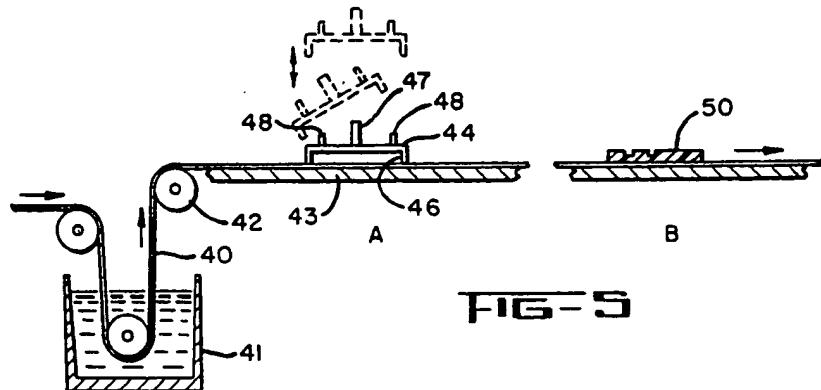


FIG-5

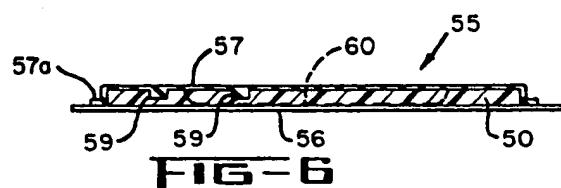


FIG-6

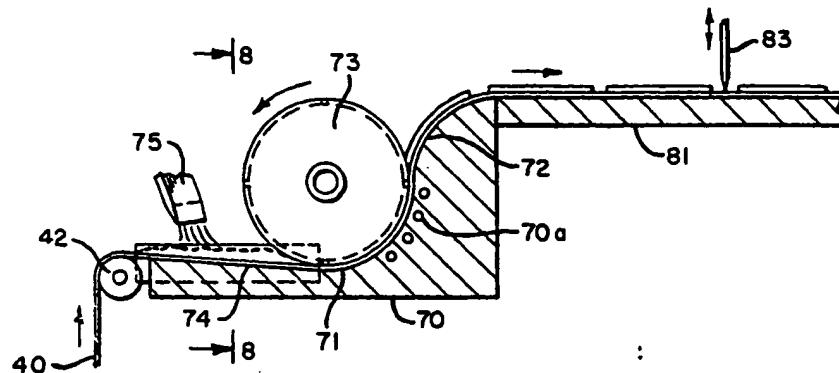


FIG-7

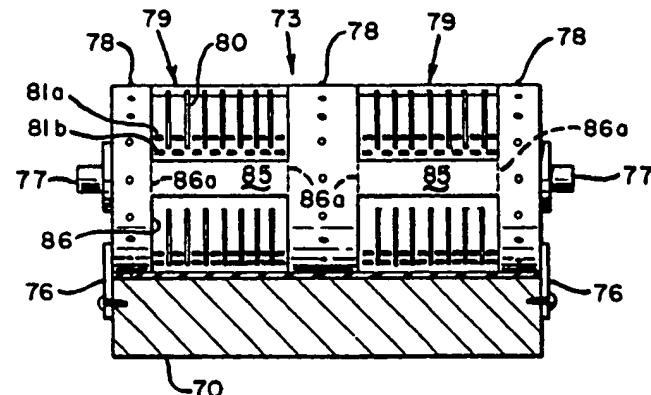


FIG-8

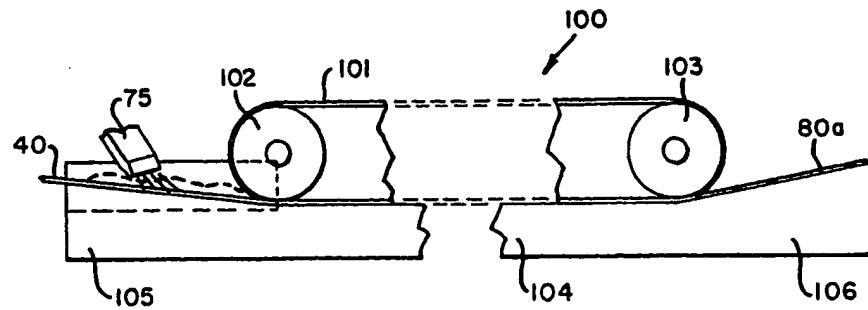


FIG-9

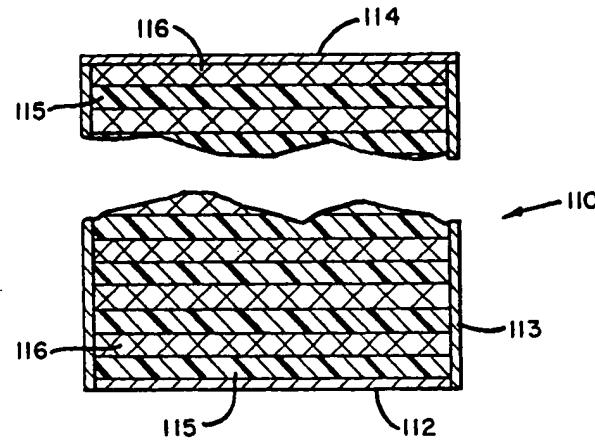


FIG-10

